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Encapsulation of endoglucanase using a biopolymer Gum Arabic for its controlled release

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Abstract

Gum Arabic, a biodegradable natural polymer was used as a matrix to encapsulate endoglucanase from *Thermomonospora* sp. The modified enzyme retained complete biocatalytic activity and exhibited a shift in the optimum temperature [50–55 °C] and considerable increase in the pH and temperature stabilities as compared to the free enzyme. Encapsulation of the enzyme also protected the activity in presence of detergents and enhanced the shelf life. A 3-fold decrease in the initial rate of reaction indicated a controlled release of the enzyme conferring properties preferred for its potential application in the manufacture of detergents.

Keywords: Endoglucanase; *Thermomonospora* sp.; Gum Arabic; Encapsulation; Eco-friendly

1. Introduction

Microencapsulation is a rapidly emerging area with multitude of applications in biotechnology, one of them being the controlled release of active biomolecule. For instance, proteolytic enzymes entrapped in liposomes are used to increase the ripening of cheese (Kirby et al., 1987; Alkhalaf et al., 1988). Microencapsulation is considered to be promising systems for oral protein drug delivery because they ensure physical protection to the encapsulated proteins against inactivation during the gastrointestinal transit. The microencapsulation of α-chymotrypsin in multiplayer alginate/protamine as a model for drug delivery system for controlled release properties has also been reported (Tiourina and Sukhorukov, 2002). An increase in transesterification rate by encapsulation of lipase has been reported (Rassy et al., 2004). Another significant example is the encapsulation of proteases in liquid detergent in order to protect the other enzymes [i.e. lipases and cellulases] from proteolysis during storage. Disruption of the microcapsules takes place on washing and releasing the enzyme. During encapsulation it is necessary that the enzyme maintains its activity while it stays inactive during processing and storage. One of the factors that govern enzyme activity is its water activity and reactivation of an enzyme can be achieved by increasing the water activity upon water uptake (Mathewson, 1998). In a controlled release system of encapsulation, degradation of matrix material occurs as a determining factor for release of the encapsulant (Pothakamury and Barbosa-Canovas, 1995; Imam et al., 1998). Thus research activities are focused on identifying matrices which impart properties for the controlled release of the encapsulated biomaterials. Extensive work has been carried out on encapsulation using synthetic polymers such as eudragit polymers, polyvinyl alcohol, polyethylene glycol, etc. However, due to the ever growing environmental concerns, uses of biopolymers such as starch, gelatin, agar, cellobiose, alginate, etc. are being favoured. Gum Arabic is a naturally produced biopolymer by *Acacia* trees growing in

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arid regions and one of the largest known emulsifiers (Whistler, 1993). Structurally Gum Arabic is a branched molecule with a main chain of (1 → 3) β-D galactopyranosyl units having side chains consisting of (1 → 3) β-D galactopyranosyl units, joined by (1 → 6) linkages (Anderson et al., 1972) with other carbohydrate contents being arabinose, glucuronic acid and rhamnose. The carboxyl groups of uronic acid are deprotonated in its normal ionized form near neutral pH values. Being a salt of polycarboxylic acid, it undergoes a degree of crosslinking if allowed to stand for a length of time (Whistler, 1993). The Gum Arabic also contains proteinaceous material covalently attached to the polysaccharide moieties (Akiyama et al., 1984).

In the present work, Gum Arabic was used as a naturally charged biodegradable polymer for the first time as a matrix to entrap thermostable endoglucanases from *Thermomonospora* sp. for their potential application in detergent and textile industries.

2. Methods

2.1. Production of endoglucanase

The production of endoglucanase was done in 500 ml Erlenmeyer flasks containing 100 ml of medium. The composition of the medium was similar to that used by Mishra et al. (1984) except for the amount of yeast extract (1%), Tween 80 (0.1%) as surfactant and cellulose paper powder (CPP) (4%) as a carbon source. Sterile 10% sodium carbonate was used to adjust the pH of the medium to 9. The inoculum (10%) was added from an inoculum flask grown for 48 h at 50 °C. The culture was grown with continuous shaking on rotary shaker at 50 °C for 120 h. The biomass was separated from the fermented broth by centrifugation and the filtrate was used as a source of endoglucanase.

2.2. Encapsulation of endoglucanase in Gum Arabic

Gum Arabic (Commercial grade Gum acacia from Burgoyne and Burbidges Company) was suspended in distilled water at 50 °C and cooled to room temperature. Endoglucanase was added to the above solution and mixed thoroughly. This mixture was kept at 4 °C for 48 h and then shifted to an air-draft oven with continuous flow of air for drying at room temperature to obtain a thin film. The film of the mixture was crushed to make a fine powder, which was used for further studies.

2.3. Scanning electron microscopy (SEM) of the encapsulated endoglucanase

Morphological examination of the fine powder of Gum Arabic and that of the encapsulated endoglucanase in Gum Arabic was carried out using SEM (Leica Stereoscan-440). Samples for SEM analysis were prepared by placing the powder on copper grids and then coating with gold.

2.4. Endoglucanase assay

The activity of endoglucanase was measured by incubating 1 ml of assay mixture containing 0.5 ml of 1% CMC and 0.5 ml of suitably diluted enzyme in 50 mM phosphate, buffer pH 5.0, for 30 min at 50 °C. Enzyme and reagent blanks were also simultaneously incubated with the test samples. The reducing sugar formed was estimated by dinitrosalicylic acid (Miller, 1959). One international unit (IU) of enzyme activity for endoglucanase was defined as the amount of enzyme releasing 1 μmol of reducing sugar from carboxy methyl cellulose per minute using glucose as standard.

2.5. Stability of endoglucanase in commercial detergents

The stability of endoglucanase in the presence of the commercial detergents Ariel, Surf Excel and Henko was investigated by incubating the enzyme in the presence of the detergent (7 mg/ml) at 40 °C. Aliquots of enzymes were removed at intervals of 10 min for 1 h and the residual activity of the enzyme was determined under standard assay conditions.

3. Results and discussion

3.1. Morphology of encapsulated endoglucanase

The endoglucanase was encapsulated in various concentrations of Gum Arabic (1–15%) and 10% was found to be most suitable. The Gum Arabic solution (pH 4.8) was mixed with the endoglucanase solution and frozen at 4 °C for 48 h for the retrogradation of the polymer. SEM of encapsulated endoglucanase in Gum Arabic showed a coating on the surface of the polymer indicating that the enzyme gets encapsulated in the scaffolds of Gum Arabic during retrogradation (Fig. 1). Previous results have shown that endoglucanase from *Thermomonospora* sp. was cationic in nature (George et al., 2001), and Gum Arabic being an anionic polysaccharide probably interacted electrostatically with the enzyme macromolecules. Encapsulation of endoglucanase in Gum Arabic provided an example of swelling-controlled system for the controlled release (Pothakamury and Barbosa-Canovas, 1995). Endoglucanase dispersed evenly throughout the matrix and was unable to diffuse to any significant extent within the matrix. But when the polymer matrix was placed in a thermodynamically compatible medium (buffer), the Gum Arabic swelled owing to absorption of buffer and endoglucanase in the swollen part diffused out of Gum Arabic. The release of enzyme from the polymer matrix has been schematically described (Fig. 2).

3.2. Properties of encapsulated endoglucanase

The activity of the encapsulated endoglucanase on dispersal in to a buffer solution was compared to that of free

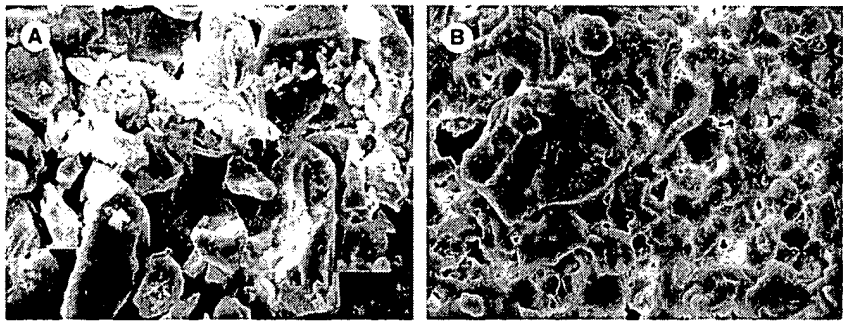

Fig. 1. Scanning electron micrograph under 1000× magnification of Gum Arabic (A) without enzyme; (B) entrapped with endoglucanase.

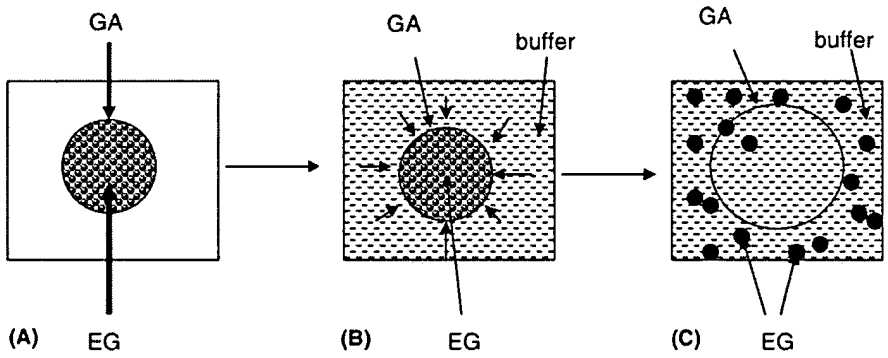

Fig. 2. Schematic representation of swelling type controlled release system of endoglucanase. (A) Endoglucanase (EG) entrapped in Gum Arabic (GA). (B) Endoglucanase entrapped in Gum Arabic placed in thermodynamically stable system: buffer diffuses into the polymer. (C) Endoglucanase released into the buffer system on swelling of the polymer matrix.

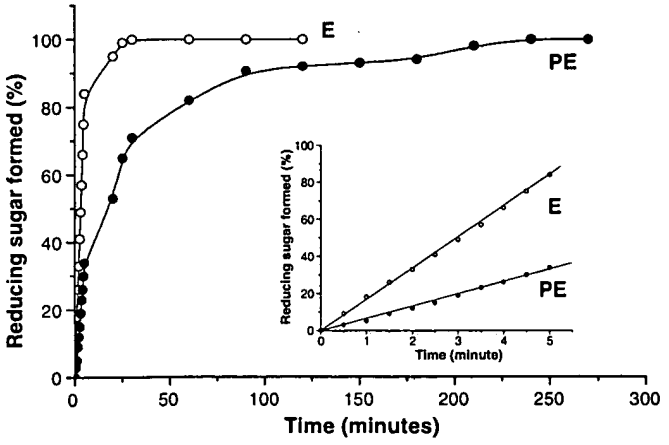

Fig. 3. Comparison of the enzymatic activity of free (E) (○) and entrapped enzyme (PE) (●). The enzyme release has been evaluated based on the formation of reducing sugar. Formation of reducing sugar monitored for a broad time course. Focus on the short times (inset). The slopes are representative of the initial rates.

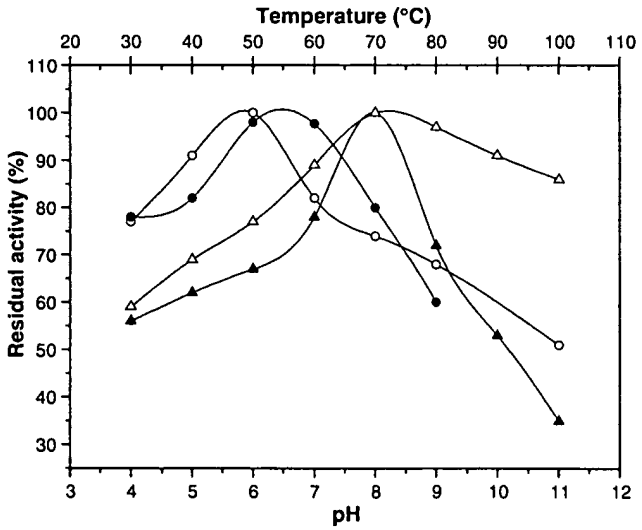

Fig. 4. The pH stability of endoglucanase action for free (○) and entrapped enzyme (●) at 50 °C. 0.05 M acetate buffer (pH 4–5), 0.05 M phosphate buffer (pH 6–7), 0.05 M Tris–HCl buffer (pH 8), 0.05 M carbonate-bicarbonate buffer (pH 9–11) were used. Temperature optima of endoglucanase action of free (△) and entrapped enzyme (▲) at various temperatures: 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C and 100 °C.

enzyme. A significant 3-fold decrease in initial rate of reaction was observed on entrapment (Fig. 3). A similar final plateau was reached indicating that the same amount of reducing sugar was liberated as a function of time indicating a controlled release of the enzyme. The encapsulated endoglucanase retained 97%, 91% and 85% activities at pH 9, 10 and 11, respectively whereas the free enzyme retained 71%, 52% and 35% of the residual activities, respectively.

Thus, pH stability of endoglucanase was considerably increased after encapsulation (Fig. 4). A shift in optimum temperature to 55 °C was observed in case of entrapped enzyme whereas the free enzyme had an optimum temperature of 50 °C (Fig. 4). The entrapped enzyme retained 100%

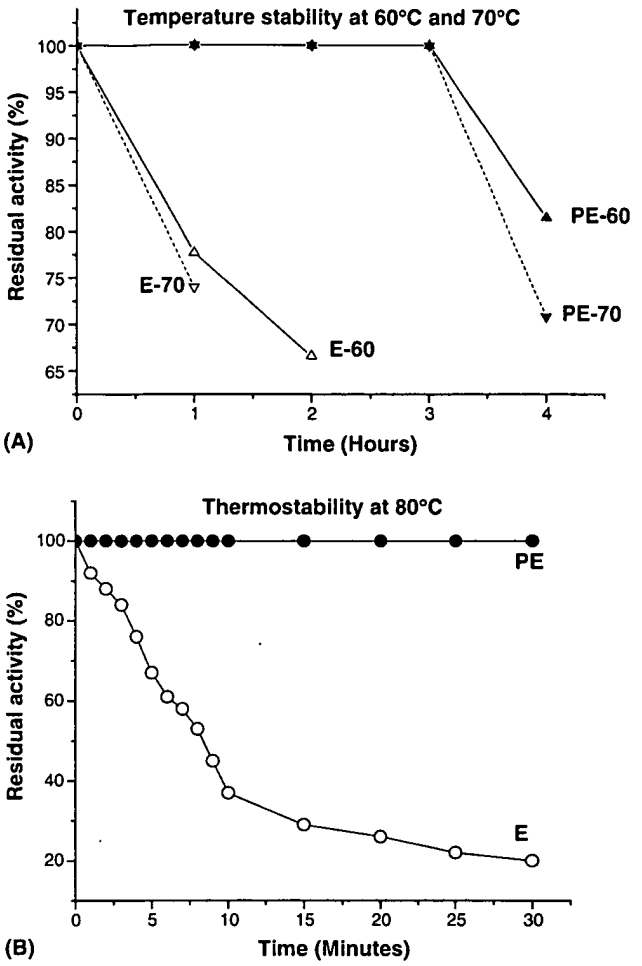

Fig. 5. Temperature stability of free (hollow symbols) and entrapped endoglucanase (solid symbols) at (A) 60 °C, (△,▲) and 70 °C (▽,▼) (B) 80 °C (○,●). 2.5 IU of endoglucanase was incubated in 0.05 M acetate buffer at various temperatures.

activity after 2 h whereas the free enzyme exhibited 74% residual activity after 1 h and negligible activity after 2 h at 70 °C (Fig. 5A). The entrapped endoglucanase retained 70% of the residual activity after 4 h at 60 °C (Fig. 5A). The polymer-entrapped enzyme exhibited a half-life of about 3.5 h at 80 °C whereas the free endoglucanase had a half-life of only 8 min (Fig. 5B). As compared to the free enzyme the encapsulated enzymes demonstrated higher temperature stability at 50, 60, 70 and 80 °C.

3.3. Stability of entrapped endoglucanase in the presence of commercial detergents

The free endoglucanase was stable for 1 h in the presence of different detergents such as Ariel, Surf Excel and Henko (retaining about 66–85% residual activities; George et al., 2001). The entrapped enzyme showed higher stability in the presence of four commercial products (Henko, Ariel, Tide and Surf Excel, which had 84–95% activity). The encapsulated endoglucanase retained 100% activity up to 60 days whereas the free enzyme exhibited 50% residual activity at the end of 15 days. The stability of the encapsulated enzyme in the presence of the commercially used detergents could be an added advantage for its application.

Few biopolymers such as starch, alginates have been used for entrapment of enzymes and proteins. Starch being neutral in charge has to be either artificially charged or can be used to entrap proteins such as amylase that binds to starch (Ongen et al., 2002). Alginates are hydrophilic polymers having ion binding properties and are used for encapsulation in combination with Ca^{2+} ions (Bregni et al., 2000). Xanthan–alginate spheres and Ca^{2+}–alginate hydrogels are used to encapsulate proteins such as urease, subtilisin, bovine serum albumin and hemoglobin. The controlled release of protein from alginate matrix was achieved by dissociation of physical hydrophobic network (Elcin, 1995; Leonard et al., 2004; Ariel et al., 2005). Subtilisin encapsulated in alginate showed enhanced temperature stability with potential for use in detergents (Ariel et al., 2005, 2006). The temperature and pH stability of urease have been shown to increase on encapsulation in xanthan–alginate spheres. The optimum temperature for the activity of urease also shifted from 50 to 60 °C (Elcin, 1995). Spherulites are also demonstrated for encapsulation of alkaline phosphatase and lipase (Bernheim-Grosswasser et al., 2000).

4. Conclusions

The encapsulation of endoglucanase was successfully carried out for the first time in a biodegradable and naturally charged polymer, Gum Arabic, with slow release of active enzyme suitable for application in detergents and textile industry. The results of the entrapment of the endoglucanase in Gum Arabic supported the hypothesis that it was possible to obtain a system, which could be triggered to degrade, when the water activity was increased upon water uptake, thus, resulting in reactivation of the entrapped enzyme. In addition, water activity could be used as a tool to inhibit the enzyme activity during storage and increase shelf life of the enzyme. In the present study, use of Gum Arabic for controlled release of endoglucanase increased thermal and pH stability with extended shelf life of the enzyme which was a preferable trait for biotechnological applications.

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